

University of Groningen

Immunological aspects of hibernation as leads in the prevention of acute organ injury

Bouma, Hjalmar Roland

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:

2013

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Bouma, H. R. (2013). *Immunological aspects of hibernation as leads in the prevention of acute organ injury*. s.n.

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Introduction

Hjalmar R. Bouma

Part of this chapter is based on:

HR Bouma, HV Carey, FGM Kroese. Hibernation: the immune system at rest? J Leukoc Biol. 2010; 88(4):619-24. Review.

HR Bouma, RJ Ploeg, TA Schuurs. Signal transduction pathways involved in brain death induced renal injury. Am J Transplant. 2009;9(5):989-97.Review.

Introduction

Mammalian hibernation represents a unique and natural model of organ preservation. The ability to hibernate increases survival during periods when food availability is low and energy demands to maintain homeothermia are high. Hibernation is an evolutionarily conserved phenomenon, which has been described in most orders of mammals (Melvin and Andrews, 2009; Heldmaier et al., 2004; Carey et al., 2003a). Hibernating animals reside most of the time in a state known as torpor, which is characterized by lowered metabolism and reduced body temperature (Figure 1.1). Such torpid states are regularly intersected by shorter periods of full restoration of metabolism and body temperature, called arousals. Despite the regular cycling between torpidity (with extremely reduced body temperature, cardiac output and tissue perfusion) the rapid rewarming during arousal does not seem to result in significant organ damage. Indeed, no gross signs of organ injury can be found in the kidneys (Sandovici et al., 2004; Zancanaro et al., 1999), gut (Fleck and Carey, 2005), lungs (Talaei et al., 2011) or brain (Arendt et al., 2003). In contrast, in humans, a reduced blood supply leading to ischemia and subsequent reperfusion as well as hypothermia that has been applied in attempts to reduce ischemic injury by means of metabolic suppression, are important factors involved in the etiology of organ injury in several clinical situations, such as transplantation medicine, major surgery, trauma, cardiac arrest and stroke.

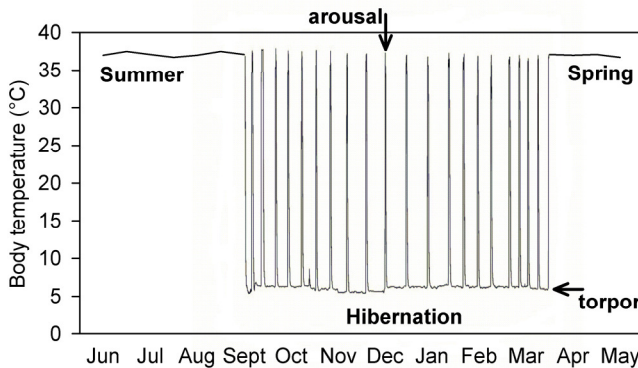


Figure 1.1: Body temperature trace of a thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*) housed in the lab at an ambient temperature of 4°C. Hibernation started in mid September and consists of torpor bouts with a body temperature $\pm 6^{\circ}\text{C}$, interspersed by short euthermic arousal periods (from: (Bouma et al., 2010a)).

Key players in the induction of acute renal injury in transplantation and major cardiac surgery

Ischemia and reperfusion are inevitable conditions during transplantation of donor organs. To date, most organs are derived from brain dead organ donors. The number of kidney transplantations performed using grafts obtained from living donors allows comparison in outcome of these with grafts derived from cardiac and/or brain death donors. Because of this practical reason, most studies performed thus far investigate the effect of donor type on graft survival in the setting of kidney transplantation. However, it is likely that injury of other organs is due to comparable if not similar molecular mechanisms. Kidneys derived from brain dead organ donors have an increased risk of delayed graft function and inferior long-term graft survival when compared to organs retrieved from living donors (Terasaki et al., 1995; Hariharan et al., 2000; Matas et al., 2002). In organ donors, the unphysiological

state of brain death induces an inflammatory response in peripheral organs (“grafts-to-be”). Brain death is most often due to cerebral hemorrhage, trauma or an anoxic event leading to the formation of edema that suppresses the blood flow and diminishes cerebral perfusion. Subsequent ischemia of the hypothalamus induces the Cushing-reflex and results in hemodynamic instability with severe hypotension and poor organ perfusion. In addition, neuronal injury induces an inflammatory response in the brain leading to endothelial injury and leakiness of the blood-brain-barrier, allowing cytokines produced in the brain to leak away into the circulation. Patients with traumatic brain injury show elevated serum levels of markers for brain injury and inflammation, including S100A9 (Kusaka et al., 2007), S100 β (Bloomfield et al., 2007; Muller et al., 2007), interleukin-6 (IL-6) (Laskowitz et al., 1998; Minambres et al., 2003), complement activation and coagulation disorders (Nekludov et al., 2007). The combination of poor tissue perfusion due to hemodynamic instability and the occurrence of a cerebral inflammatory response is likely to be involved in the induction of an inflammatory response in peripheral organs. Subsequent preservation of these organs leads to exaggeration of this inflammatory response due to ischemia and reperfusion. This, together with direct injury caused by ischemia/reperfusion, damages to the graft-to-be (Kusaka et al., 2007; Weiss et al., 2007). Following transplantation, the reduced functioning nephron mass leads to exhaustion of the remaining nephrons due to hyperfiltration, in order to try to meet the metabolic needs (Terasaki et al., 1994), which in turn results in accelerated dysfunction (Heemann et al., 1994). Marginal tissue perfusion leading to ischemia/reperfusion is not only an inevitable event occurring in the setting of organ transplantation, but might also be involved in the etiology of renal injury after cardiac surgery. Renal injury affects up to 30 % of the patients following cardiac surgery using cardio-pulmonary bypass (CPB) (Loef et al., 2005; Karkouti et al., 2009; Loef et al., 2009). Although this form of renal dysfunction is usually transient and self-limiting, the temporary occurrence of renal dysfunction is associated with increased mortality during the in-hospital stay and long term follow up (\pm 100 months) (Loef et al., 2005). In addition to ischemia/reperfusion, inflammation and hypothermia are also suggested to be involved in the etiology of renal injury following CPB-assisted surgery (Asimakopoulos, 2001; Holmes et al., 2002; Caputo et al., 2002; Kourliouros et al., 2010).

Hypothermia is used to prevent tissue damage during ischemia

Decreased tissue perfusion becomes critical once it shortfalls tissue metabolism. Various attempts have been made to reduce tissue metabolism, oxygen consumption and hence, the occurrence of ischemia. An example of such a strategy is therapeutic hypothermia. To date, therapeutic hypothermia is used to limit organ injury under the ischemic conditions of organ preservation in transplantation medicine, during major (cardiac) surgery and sometimes following cardiac arrest (Terasaki et al., 1996; Arrich et al., 2009; Lee and Asare, 2010). However, prolonged cold storage of transplantation organs leads to tissue damage and inferior long-term graft survival (Salahudeen, 2004). In cardiac surgery, therapeutic hypothermia may prevent ischemic brain injury by reducing metabolic and oxygen consumption (Nussmeier, 2005), but its major drawback is the direct induction of cellular injury, illustrated by the increased incidence of kidney failure following hypothermic cardiac surgery (Asfour et al., 1996; Kourliouros et al., 2010).

Mechanistic insights into tissue injury induced by ischemia and hypothermia

Tissue injury during (cold) ischemia is amongst others caused by adenosine triphosphate (ATP)-depletion, accumulation of hypoxanthine, loss of the Na⁺/K⁺ pump-activity, cell swelling, and increases in cytosolic calcium (Salahudeen, 2004). While ATP is consumed in order to maintain electrolyte homeostasis, a low temperature decreases ATP synthesis in the absence of oxygen. A shortage in either glucose or oxygen in a tissue (e.g. due to a reduced blood supply), might lead to a reduced production of ATP. When the shortfall in energy production cannot be compensated for by decreased ATP consumption, cellular homeostasis is disrupted and cell death is induced (Hochachka, 1986; Boutilier, 2001; Lodish et al., 2008; Aslami and Juffermans, 2010; Storey, 2010). Moreover, ischemia induces a switch to anaerobic metabolism which affects the cellular pH through the accumulation of lactate. This in turn results in lysosomal and mitochondrial instability, the latter contributing to progressive cellular energy depletion (Salahudeen, 2004). The lack of ATP will ultimately result in impairment of the Na⁺/K⁺-ATPase, permitting intracellular accumulation of sodium and water and hence cell swelling. Mitochondrial instability combined with decreased functioning of cellular reactive oxygen species (ROS)-scavengers facilitates the accumulation of intracellular ROS (Brinkkoetter et al., 2008), which may result in endothelial damage. These conditions favor an inflammatory reaction in the graft, as reflected by the activation of endothelial adhesion molecules, e.g. intracellular cell adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1) and selectins (Jassem et al., 2003).

Mammalian hibernation

A potentially safer method of reducing metabolism might be found in nature: *mammalian hibernation*. By hibernating, small animals can conserve up to 88 % of the energy they would otherwise need to maintain normothermic metabolism and body temperature during winter (Wang, 1979; Hampton et al., 2010; Heldmaier et al., 2004; Milsom et al., 1999). Entrance into hibernation is characterized by a drop in metabolic rate to 1-5 % of euthermic rates and a reduction of body temperature to a few degrees above the ambient temperature (Storey, 1997; van Breukelen and Martin, 2002; Carey et al., 2003a). Hence, typical body temperatures during deep torpor are $\pm 0-4^{\circ}\text{C}$ (Kenagy et al., 1989), while the lowest recorded body temperature during deep torpor is -2.9°C in Arctic ground squirrels (*Urocitellus parryii*; (Barnes, 1989). Profound changes in physiology are observed during torpor. In deep torpor, ground squirrels reduce their heart rate from 350-400 to 5-10 beats per minute, respiration is depressed from approximately 40 breaths per minute to less than one breath per minute and renal function is severely decreased (Zancanaro et al., 1999; Carey et al., 2003a; Storey, 2010). A deep torpor may last from a few days up to 35 days (depending on the species) and are interspersed by short periods of arousal in which metabolic rate and body temperature rapidly increases to reach euthermic values, which may last one to two days (Twente and Twente, 1965; Heldmaier et al., 2004). In addition to multi-day bouts of torpor during hibernation, in other species torpor may occur on a daily basis with bouts that last less than 24 hours and is characterized by body temperatures that generally remain $\geq 18^{\circ}\text{C}$ (Heldmaier et al., 1999; Heldmaier et al., 2004; Geiser, 2004). Interestingly, daily torpor is preserved among many small mammals and can also be observed in non-human primates such as the fat-tailed dwarf lemur (*Cheirogaleus medius*; (Dausmann et al., 2004), thus underlining the evolutionary conservation of torpor behavior.

The hibernating immune system

The first line of defense against invading pathogens consists of skin and mucosal barriers, followed by the innate immune system, which consists of the complement system, granulocytes (neutrophils, basophils and eosinophils) and monocytes/macrophages (e.g. Kupffer cells and alveolar macrophages). Defense against pathogens by the innate immune system is governed by inducing cell lysis following phagocytosis (internalization of microbes) or extracellular by releasing toxins such as reactive oxygen species (ROS). Antigen-specific immune reactions can be induced by the adaptive immune system, in which antigen presenting cells such as monocytes, neutrophils or dendritic cells activate lymphocytes to induce either a cellular (cytotoxic) or humoral (antibody-mediated) response. Further, several populations of “suppressor” or “regulator” cells serve a role to limit the activity of the immune system and prevent organ injury due to inflammation. Although there are few studies that have examined in detail the effect of hibernation on the immune system, available data indicate that hibernation in mammals decreases the function of both the innate and adaptive immune system. One of the most striking and pronounced changes in the immune system of torpid hibernators is the reduction in numbers of circulating leukocytes (leukopenia). Numbers of circulating leukocytes drop by $\pm 90\%$ during torpor in all hibernating mammals studied so far, including the European hamster (*Cricetus cricetus*; Reznik et al., 1975), the hedgehog (*Erinaceus europeus* L.; (Suomalainen and Rosokivi, 1973), the European ground squirrel (*Spermophilus Citellus*; (Bouma et al., 2010c), the Arctic ground squirrel (*Uroditellus parryii*; (Toien et al., 2001) and the thirteen-lined ground squirrel (Spurrier and Dawe, 1973; Frerichs et al., 1994). The leukopenia is reported to affect granulocytes (neutrophils, eosinophils and basophils), lymphocytes and monocytes, while the $\pm 10\%$ remaining leukocytes in the blood during torpor consists mainly of neutrophils (90%) and lymphocytes (9%) (Szilagyi and Senturia, 1972). During arousal, the numbers of neutrophils and monocytes increase rapidly to summer (euthermic) levels, whereas the numbers of lymphocytes increase to only $\pm 50\%$ of summer values (Suomalainen and Rosokivi, 1973). The absence of circulating neutrophils and monocytes may result in a significant reduction of acute inflammatory responses and a defective clearance of microbes, while the reduction in lymphocytes will strongly affect immune surveillance leading to impaired cellular and humoral immune responses. A reduced function of the innate immune system is suggested by the observations that during torpor, the febrile response to injection of lipopolysaccharide (LPS) is delayed until the next arousal (Prendergast et al., 2002), macrophages produce less TNF- α (Novoselova et al., 2000), and complement level is decreased during torpor (Maniero, 2002). A diminished function of adaptive immunity on the other hand is suggested by the absence of rejection of skin allografts transplanted into torpid thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*) until the end of the hibernation season (in spring) and are thus surviving 3-4 times longer than allografts transplanted to summer animals (Shivatcheva, 1988). Finally, the antigen-induced antibody production is severely reduced in hibernating ground squirrels (*Ictidomys tridecemlineatus*) and hamsters (*Mesocricetus brandti*), as compared to summer animals (Sidky et al., 1972; Burton and Reichman, 1999). Thus, specific alterations in the immune system occur during hibernation and seem to lead to an overall reduced immune function.

Hibernation as natural model of organ preservation

As described above, hibernating animals survive periods of physiological extremes without signs of organ injury, while many of the physiological extremes of hibernation are expected to lead to organ damage in non-hibernating animals. Specific alterations in cellular respiration might allow them to preserve homeostasis, while a reduced immune function might prevent the induction of an inflammatory response and exaggeration of tissue injury. Since hibernators have an increased resistance to ischemia/reperfusion, survive periods of torpor with an extremely low body temperature without signs of organ injury and several studies suggest a reduced immune function, these animals might have a superior outcome following CPB as compared to non-hibernating animals as ischemia/reperfusion, hypothermia and inflammation seem to be the key factors involved in the induction of acute kidney injury by CPB. Therefore, unraveling the mechanisms that underlie these features of hibernators and those that can mimic such a state in non-hibernating animals, might lead to the discovery of pharmacological targets to limit renal injury induced by CPB and improve survival following surgery.

Scope of this thesis

This thesis aims at exploiting natural hibernation to protect organs from acute injury during transplantation or cardiac surgery. Unraveling the mechanisms that play a role in the induction of torpor and its adaptations such as immunosuppression, might enhance our ability to translate this information into biomedical applications, such as better use of clinical hypothermia and development of therapies for other disorders in which metabolic imbalance or inflammation play important roles. To this end, we explored the mechanisms that are involved in the induction of renal injury following brain death and cardiopulmonary bypass by using literature research, animal models and a retrospective patient study. Next, we explored adaptations of mammalian hibernators that allow them to safely survive periods of torpor and arousal, in particularly the reduced immune function. Finally, we performed a literature review to inventorise current strategies to induce a torpor-like state in non-hibernating species and studied the effects of pharmacologically induced torpor on the immune system.

